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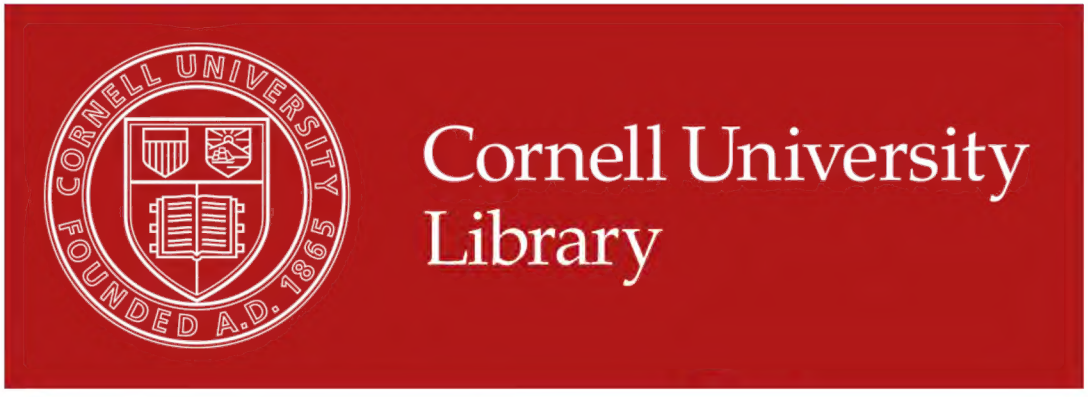

A STUDY OF THE

FERTILIZATION OF ALFALFA FLOWERS

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C. T.

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INTRODUCTION.

In 1907 a study of the factors controlling the pollination of alfalfa flowers was begun by the writer, who was then located at Pullman, Washington, in charge of the cooperative experimental work with forage crops being carried on there by the U. S. Department of Agriculture and the Washington State Experiment Station. The investigation was begun in 1907 and was continued during the summers of 1908, 1909 and 1910. Most of the work was done on the experimental plots at Pullman, Washington, though considerable of the work done at Pullman was duplicated in alfalfa fields near Chinook, in northern Montana. Chinook is located in one of the most important alfalfa seed producing districts in the Northwest.

The suggestion was made to the writer in July, 1907, by members of the Office of Forage Crop Investigations of the U. S. Department of Agriculture, that the relationship of the factors controlling the pollination of alfalfa flowers to the development of the alfalfa seed crop was a problem that had never been thoroughly investigated. At that time there was but a comparatively meager amount of information

on this subject, and practically no investigations that have been recorded had been carried on in the United States. Since 1907 several other investigators, including three members of the Office of Forage Crop Investigations, have been making a study of the pollination of alfalfa flowers. This report, however, contains only a discussion of the results which were obtained by the writer in the work conducted at Pullman, Washington, and at Chinook, Montana.

DESCRIPTION OF THE ALFALFA FLOWER.

Before the bud of the alfalfa flower has developed into a blossom the broad standard petal is wrapped closely around the wing petals, which in turn tightly enclose the keel. As the flower develops, the standard unfolds and at the same time curves backwards, while the two wing petals also unfold from over the keel. The two keel petals, however, remain joined together to about one-half the distance from the tip back toward their base. The stigma and the stamens are so situated that they are closely enveloped in the tip of the keel, with little chance for any pollen from another flower to reach the stigma as long as the keel petals are united.

If the tip of a pencil, toothpick, grass

stem, or some other similar object, be gently pushed down between the standard and the keel in such a way that the latter is pressed backwards, the strain will disunite the two petals of the keel. As soon as the restraining force of the united keel petals is removed the pistil and the staminal column instantly spring into the new position up against the standard. The flower is now "tripped". When the flowers are tripped by honey-gathering insects both anthers and stigma usually come into contact with the body of the insect during the process. As the pollen adheres to different portions of the insect, there is abundant opportunity for the flowers to become cross-fertilized with pollen from the flowers of other plants.

CONDITION OF FLOWERS IN THE ALFALFA FIELDS.

Before any experiments had been planned or executed an examination was made of the condition of the alfalfa flowers in the fields near Pullman.

It was found that a large proportion of the flowers had not been tripped and that the pistils of most of the tripped flowers were in a fresh, turgid condition; on the other hand, it was observed that many flowers had been tripped and that most of the tripped flowers were more or less wilted.

During bright, warm days domestic honey bees and other honey gathering insects were more or less abundant in the fields. It was not known, however, whether it was these insects or some other agencies which had caused the flowers to become tripped. Furthermore, there was considerable difference of opinion in regard to the self-fertility of the alfalfa flower.

DESCRIPTION OF EXPERIMENTS AND DISCUSSION OF THE RESULTS.

Comparative Proportions of Flowers Producing Seed under Natural Conditions when Protected from Insects and when Artificially Tripped.

In order to find out something in regard to the manner in which the alfalfa flowers become tripped, and what the effect of excluding insects from the plants is, an experiment was planned and carried out in 1907 and was repeated in 1908, 1909 and 1910. A considerable number of alfalfa plants were used in carrying out this experiment.

The stems of each plant were divided into three groups each having an approximately equal number of flowers. The flowers on the first group of branches were left to develop under natural conditions. The other two groups of branches were enclosed in a small tent or cage made of a fine-meshed mosquito netting or tarlatan having about twenty-four meshes to the inch, which effectually excluded all honey gathering insects. The flowers on one of these groups of branches were left to develop with no other treatment than to exclude insects. On the third group of branches a number of flowers were artificially tripped by means of a toothpick, green alfalfa stem or

some other similar object. In carrying out this experiment the flowers which were tripped artificially were not cross-pollinated. As a single toothpick or grass stem was ordinarily used to trip a number of flowers on one plant, many of the flowers might have been fertilized with pollen carried on the toothpick from one flower to another on the same plant.

In carrying out the experiments in 1907 the raceme was taken as a unit and no count was made of the actual number of flowers that were on each raceme. From 11 racemes which developed under natural conditions, 49 pods developed; from 36 racemes of flowers from which insects were excluded, 28 pods developed; and from 12 racemes on which the flowers were artificially tripped, 25 pods developed. These results obtained in 1907 substantially agree with those obtained in the three succeeding years.

The results obtained in 1908 are given in Table I.

Table I.

(Results of Alfalfa Tripping Experiments in 1908.)

	Total no. flowers.	No. pods developed.	Total no. seeds	% of flowers developing pods	No. seeds per pod.
Outside netting; conditions natural	869	207	574	23.8	2.77
Inside netting; flowers not tripped	912	26	32	2.8	1.23
Enclosed in netting; tripped artificially	576	148	205	25.6	1.38

In 1909 the experiment was carried out in the same way at Pullman, Washington, and also at Chinook and Havre in northern Montana. The results are given in Table II.

Table II.

Pods and Seeds Forming Outside Netting, Inside Netting, and from Flowers Artificially Tripped.

Outside Netting, Conditions Natural.

	No. of plants used.	No. of racemes	No. of flowers	No. of pods formed	No. of seeds	Per cent of flowers developing pods	Seeds per pod.
Pullman -	15	90	1468	480	1730	32.69	3.60
Chinook -	10	76	944	143	320	15.14	2.23
Havre -	9	41	366	51		13.93	
	34	207	2778	674	*2050	24.25	*3.29

Inside Netting, Insects Excluded.

Pullman -	15	90	1500	131	357	8.73	2.72
Chinook -	10	104	1186	138	282	11.63	2.04
Havre -	9	60	535	30		5.60	
	34	254	3221	299	*639	9.28	*2.37

Enclosed in Netting, Flowers Artificially Tripped.

Pullman -	15	101	1379	599	1783	43.43	2.97
Chinook -	10	64	830	370	681	44.57	1.84
Havre -	9	46	337	104		30.86	
	34	211	2546	1073	*2464	41.75	*2.54

* Numbers marked with * refer only to results obtained at Pullman and Chinook.

In 1910 the same experiment was repeated at Pullman, Washington. The results are given in Table III.

Table III.

(Results of Alfalfa Tripping Experiment - 1910.)

	Total no. flowers.	No. pods developed.	No. mature seeds:	% of flowers developing pods:	No. mature seeds per pod
Outside netting; conditions natural	228	30	38	13.15	1.26
Inside netting; flowers not tripped	354	15	24	4.24	1.6
Inside netting; flowers artificially tripped	640	140	171	21.87	1.22

The comparatively small percentage of the flowers outside of the netting and of the flowers artificially tripped, which developed into pods, was appartently due to the droughty conditions existing at the time this experiment was conducted in 1910. The comparatively small number of seeds per pod is probably due to the same cause.

The results obtained in 1908, 1909 and in 1910 are combined in Table IV.

Table IV.

Results of Alfalfa Tripping Experiments - 1908, 1909 and 1910.

Outside netting.

	Total no. of flowers	No. of pods	Total no. of seeds	Per cent of flowers developing pods	No. seeds per pod.
1908 - Pullman -	869	207	574	23.80	2.77
1909 - Pullman -	1468	480	1730	32.69	3.60
Chinook -	944	143	320	15.14	2.23
Havre -	366	51		13.93	
1910 - Pullman -	228	30	38	13.15	1.26
	3875	911	*2668	23.50	*3.09

Inside netting - not tripped.

1908 - Pullman -	912	26	32	2.80	1.23
1909 - Pullman -	1500	131	357	8.73	2.72
Chinook -	1186	138	282	11.63	2.04
Havre -	535	30		5.60	
1910 - Pullman -	354	15	24	4.20	1.60
	4487	340	*695	7.57	*2.24

Inside netting - artificially tripped.

1908 - Pullman -	576	148	205	25.6	1.38
1909 - Pullman -	1379	599	1783	43.43	2.97
Chinook -	830	370	681	44.57	1.84
Havre -	337	104		30.86	
1910 - Pullman -	978	258	392	26.30	1.51
	4100	1479	*3061	36.07	*2.22

* The results obtained at Havre in 1909 are not included for the reason that the seed did not mature before frost.

The results of these experiments show that when honey gathering insects were excluded from the flowers the usual result was to cause a decided decrease in the number of pods and seeds formed. The only marked exception to this rule occurred at Chinook, Montana, in 1909, when excluding insects did not greatly reduce the number of pods formed. The reasons why such a comparatively large proportion of the flowers, which were enclosed in netting, at Chinook produced pods and seeds will be discussed on page of this report.

An examination of the preceding table shows that 23.50 per cent of the 3875 flowers which developed under natural conditions produced pods. Of the 4487 flowers which were inclosed in netting tents to exclude honey gathering insects only 7.57 per cent produced pods or seed. When the 4100 flowers from which insects were excluded were artificially tripped the percentage of flowers which produced seed was increased to 36.07 per cent. This increase in the number of pods and seeds formed was very marked in each year and at each locality where the experiment was conducted.

When the flowers were allowed to develop under natural conditions an average number of 3.09 well matured seeds per pod were formed. When the insects were excluded the number of seeds per pod was reduced to 2.24; and when the flowers were artificially pollinated with

no opportunity for pollen to be carried from the flowers of one plant to those of another, the number of seeds per pod was 2.22,--about the same as when the flowers under netting were not artificially tripped. These figures indicate that flowers fertilized with pollen from other plants produce more seed than if they are fertilized with pollen from the same plant. Another experiment, discussed on page , brings out this point more clearly.

Effect of Protecting the Alfalfa Flowers from Sun and Wind by a Single Thickness of Mosquito Netting, but with Provision for Ready Access of Insects.

In carrying out the experiments recorded above those plants or portions of the plants from which insects were excluded were protected by a single thickness of fine mosquito netting, arranged in tent-like form by placing it over pieces of wood from four to six feet long. It was observed when the experiments were being carried out that on the different plants those flowers which were enclosed in the covering made by a single thickness of mosquito netting remained in bloom longer, and that the petals seemed to be larger, than on those flowers which were not enclosed. As it seemed possible that the effect of the slight shade, or possibly the breaking of the force of the wind, might influence the development of seed, an experiment was carried out, in 1908, to obtain some information in regard to this point.

Several plants were selected and a portion of each plant was enclosed in a netting tent, as in the other experiment; the remaining portion of each plant selected was not enclosed, but was protected on three sides and partially from above by one thickness of netting, which was between the plant and the sun, and also between the plant and the prevailing winds, yet did not prevent the access of bees and other insects. The results of this experiment are given in Table V.

Table V.

Effect of a Covering of One Thickness of Netting over the Flowers.

Plant no.	No. of flowers	No. pods developed.	No. seeds	Per cent of flowers developing seeds.	No. seeds per pod.
Shaded by one thickness of netting, but not enclosed.					
03	160	35	53	21.8	1.51
03-a	71	21	71	29.5	3.38
41	112	31	88	27.6	2.83
89	99	22	43	22.2	1.95
89-a	71	18	41	25.3	2.27
	513	127	296	24.7	2.33
Entirely enclosed in mosquito netting.					
03	141	14	19	9.9	1.35
03-a	99	7	19	7.0	2.71
41	132	4	5	3.0	1.25
89	105	2	3	1.9	1.5
89-a	43	0	0	0.0	0.00
	520	27	46	5.1	1.7

As shown in Table V, of those flowers not entirely enclosed in netting, but which were shaded by the netting, 24.7 per cent produced pods and seed. On other plants used in experiments in 1908, 27.8 per cent of the flowers which were not enclosed or shaded by netting produced pods and seed. According to these results there was no appreciable effect caused by the screen of one single thickness of mosquito netting on the development of seed on these plants.

Comparative Number of Pods and Seeds Forming under Natural Conditions from Flowers which have been tripped and from Flowers which have not been tripped.

The figures given in the preceding tables show that when alfalfa flowers are screened from the visits of honey-gathering insects, the effect is to greatly reduce the percentage of pods that are formed from the flowers. However, only a portion of the flowers used in the experiments described above, which were left to develop under natural conditions, were visited or tripped by honey-gathering insects.

In order to determine what proportion of the flowers which are actually tripped produce seed, a number of racemes of flowers on different plants were selected, in 1908, on which a portion of the flowers had been tripped by natural causes. On the calyxes of those flowers which had been tripped a small quantity

of an insoluble drawing ink was applied. After the tripped flowers had been marked the racemes were enclosed in netting to prevent the access of any more insects. Table VI shows the number of pods and seed which developed from tripped and untripped flowers.

Table VI.

Showing Number of Flowers Tripped under Natural Conditions.

Raceme number	Number flowers in raceme	Number flowers tripped	No. flowers not tripped when inclosed	Number pods developed from tripped flowers	No.pods developed from fls.not tripped when enclosed	Total no. seeds on cluster from tripped flowers	Total no. seeds developed from fls.not tripped when enclosed
1	11	4	7	2		2	
2	11	8	3	5		33	
3	16	11	5	8		55	
4	8	6	2	3		2	
5	17	11	6	7	1	11	1
6	10	52	5	4		14	
7	18	10	8	2		2	
8	9	9	0	8		33	
9	11	6	5	5		11	
10	13	8	5	4		3	
11	15	8	7	6		25	
12	11	7	4	0	2	0	2
Total	150	93	57	54	3	191	3

Percentage of tripped flowers developing pods - - - - - 58.00
Percentage of flowers not tripped when enclosed developing pods- 5.20
Number seeds per pod from tripped flowers - - - - - - - 3.53
Number seeds per pod from flowers not tripped when enclosed - 1.00

The figures given in Table VI show that 58 per cent of the flowers which had been tripped by insects or by some other natural agency produced seed; whereas only 5.2 percent of those flowers not tripped when enclosed in netting produced seeds. The flowers which were tripped when enclosed in the netting produced 3.53 seeds per pod, while the three flowers which were not tripped when enclosed in netting, which produced seeds, produced only one seed per pod. These results agree with those shown in Table IV. Table VI also indicates that most of the alfalfa seed produced at Pullman, Washington, in 1908, developed from flowers which had been tripped by honey-gathering insects; and that but a small proportion of the flowers which were not visited by honey-gathering insects produced seeds.

Effect of Fertilization with Pollen of Different Degrees of Relationship.

As the results of previous experiments indicated that the manner in which a flower is fertilized has something to do with the percentage of pods and the number of seeds formed per pod, an experiment was planned and carried out in 1909 to obtain more definite information in regard to this problem. On six selected plants a portion of the flowers were tripped with a rough piece of string, each flower being tripped alternately with the flowers of one of the other plants. In this way there was opportunity to fertilize each flower with pollen from another plant. On three of these plants the remaining flowers that had been tagged were tripped with pieces of grass or alfalfa stems. As a separate stem was used for each flower, each stigma received pollen only from the same flower. On each of the other three plants, a portion of the flowers were tripped by pressure from the outside in such a way that no external object came in contact with the stigma, thus preventing cross fertilization. The results are presented in tables VII and VIII.

Table VII.

Flowers fertilized with pollen from other plants.				Flowers tripped with separate stems; fertilized with pollen from same flower.		
Plant number	No. of flowers	No.of pods	No.of seeds	No. of flowers	No.of pods	No.of seeds
52	55	42	174	47	20	36
53	47	36	130	48	19	25
55	52	13	34	48	7	11
	154	91	358	143	46	72
Percentage of flowers developing pods - -			59.09	Percentage of flowers developing pods - -		32.16
Seeds per pod - - - - -			3.71	Seeds per pod - - - - -		1.56

Table VIII.

Flowers fertilized with pollen from other plants.				Flowers tripped by outside pressure; fertilized with pollen from same flower.		
Plant number	No. of flowers	No.of pods	No.of seeds	No. of flowers	No.of pods	No.of seeds
54	42	34	134	59	30	40
56	48	26	66	37	18	28
57	51	35	125	51	15	34
	141	95	325	147	63	102
Percentage of flowers developing pods - -			67.37	Percentage of flowers developing pods - -		42.85
Seeds per pod - - - - -			3.42	Seeds per pod - - - - -		1.61

In both tables the flowers fertilized with pollen from separate plants produced a larger percentage of pods and a larger number of seeds per pod than the flowers fertilized with their own pollen. The results of this experiment may

perhaps be subject to the criticism that the flowers fertilized with their own pollen should have been tripped with pieces of rough string, as the flowers fertilized with pollen from separate plants were, instead of with a smooth plant stem or by pressure; for the irritation of the stigma with the rough string may possibly have had some influence upon the development of the flowers into pods.

In 1910 an experiment was conducted in which flowers which were fertilized with pollen from other plants, as in the experiment just described, were compared with flowers which were artificially fertilized with pollen from other flowers of the same plant. The flowers fertilized in each of these two ways were tripped in the same manner, by using a piece of alfalfa stem wound with thread. The results of this experiment are given in Table IX.

Table IX.

Plants enclosed in netting; flowers tripped, with alfalfa stem wound with thread, alternately with flowers of another plant:-

Plant number	Number racemes	Number flowers	Number pods	Number mature seeds	Total Number seeds
1	3	38	18	27	39
2	4	50	21	51	53
3	4	54	21	110	113
4	4	52	4	7	7
5	4	48	24	5	49
6	4	45	10	3	15
7	4	51	20	18	58
	27	338	118	221	334

Percentage flowers producing pods--- 34.8

Number mature seeds per pod---------- 1.87

Total number seeds per pod---------- 2.83
(Including immature seeds)

Flowers enclosed in netting; tripped with alfalfa stem wound with thread; fertilized with pollen from other flowers of same plant.

Plant Number	Number racemes	Number flowers	Number pods	Number mature seeds	Total number seeds
1	4	58	5	7	7
2	3	41	11	26	26
3	5	56	13	16	19
4	4	72	14	22	22
5	4	56	30	27	73
6	2	23	2	5	5
7	5	51	9	6	10
	27	357	84	109	162

Percentage flowers producing pods ------23.5

Number mature seed per pod---------- 1.3

Total number seeds per pod---------- 1.93

The smaller percentage of flowers which produced pods and the smaller number of seeds per pod, from both cross fertilized and self fertilized flowers, in 1910 than in 1909, was apparently due to the drier weather conditions which existed in 1910.

The percentage of flowers producing pods, the number of mature seeds per pod, and the total number of seeds per pod, is about 50 per cent more in each instance in 1910 where the flowers were fertilized with pollen from other flowers of the same plant.

The results of this experiment agree substantially with the results of the experiment conducted in 1909. In both instances the flowers which had been fertilized with pollen from other plants produced a larger proportion of pods and more seeds per pod than did the flowers which had been fertilized with their own pollen or with pollen from other flowers on the same plant.

Another experiment was carried out in 1910 to obtain some information in regard to the relative effect of fertilizing with pollen from other flowers of the same plant compared with the effect of fertilizing with pollen from the same flower. The flowers that were fertilized with pollen from other flowers of the same plant were tripped with a piece of medium coarse wrapping twine, a single piece of twine being used to trip all of the flowers tripped on one plant, in order that pollen might be carried from one flower to another. The flowers fertilized with their own pollen were tripped by using a separate piece of twine for each flower. The hands were sterilized after tripping one flower, before tripping the next, in order to prevent pollen being carried from one flower to another. Both methods were used on flowers of each of several different plants.

Table X.

Comparative effect of pollen from same flower and of pollen from other flower of the same plant, flowers enclosed in netting; fertilized with pollen and other flowers on the same plant.

Plant number	Number racemes	Number flowers	Number pods	Number mature seeds	Total number seeds
1	5	34	9	13	18
3	6	69	9	1	18
5	6	66	35	36	90
7	6	59	17	13	21
	23	228	70	63	147

Percentage of flowers developing pods---30.7

Number mature seeds per pod---------- .9

Total number seeds per pod---------- 2.1

Flowers enclosed in netting; fertilized with pollen from the same flowers.

Plant number	Number racemes	Number flowers	Number pods	Number Mature Seeds	Total number seeds
1	5	43	9	9	11
3	3	29	2	0	2
5	6	61	42	23	55
7	5	50	19	24	34
	19	183	72	56	102

Percentage flowers developing pods -----39.3

Number mature seeds per pod---------- .77

Total number seeds per pod---------- 2.19

The figures presented in Table X do not show a very mark-

ed difference in the effect of the pollen from separate flowers of the same plant, compared with the effect of the pollen from the same flower. The average percentage of flowers producing pods is slightly less, while the number of mature seeds per pod is slightly more from the flowers fertilized with pollen from other flowers of the same plant, than from flowers of the same plant, than from flowers fertilized with their own pollen. The total number of seeds per pod developing from the two different methods of fertilization is almost exactly the same.

Pods Formed When Stigma and Pollen Do Not Come Into Contact With Any Object When The Flower is Tripped.

Two experiments were performed in 1910, to find out what the effect would be when the flower is tripped, but when the stigma does not come into contact with the surface of the object tripping the flower or in contact with the standard of the flower. In the first experiment the standard was removed; then the flower was carefully tripped in such a way that the stigma and pollen did not come in contact with any object during the process. In the second experiment, the flowers were tripped by cutting the lower part of the anterior side of the keel with a sharp razor, so that the tip of the keel remained enclosing the anthers and stigma moving up with the staminal column when the flowers were tripped, thus preventing the stigma and anthers from emerging from the inside of

the keel or from coming into contact with the standard or any other object. For the sake of comparison other flowers on the same plants were tripped by using a single alfalfa stem for all the flowers on one raceme. Other flowers which had been tripped naturally were also marked. A number of flowers which were untripped at the time when the flowers were inclosed in netting were also marked. Five plants were used in this experiment. The results are presented in Table Xl.

Table Xl.

Pods developing from flowers tripped without pistil and stamens coming into contact with any object.

	Total number flowers	Number pods found	Percentage of flowers developing pods.
Standard removed before tripping	113	16	14.15
Tripped by cutting lower part of keel, stigma and pollen remain in tip of keel	76	10	13.16
Flowers tripped with stem alfalfa plant	120	53	41.73
Flowers tripped naturally	178	93	52.24
Flowers not tripped	358	18	5.02

Table Xl shows that when the flowers were tripped without bringing the stigma and pollen into contact with any

object, a comparatively small proportion of the flowers produced pods. In other words, this experiment indicates that when the staminal column only moves from its original position to the curved position it assumes when tripped, without rubbing the stigma and pollen against the object tripping the flower or the standard. The percentage of pods produced is much less than when the flowers are tripped in the ordinary way.

Pods and Seed Produced From Automatically Tripped Flowers.

The term automatically tripped is applied to an alfalfa flower which has become tripped without the aid of insects or any other external agency.

The results of the experiments which have been recorded in this report indicate that the presence of honey gathering insects, which trip the flowers that they visit, under at least some conditions is essential in alfalfa fields where seed is to be produced. In the fields about Pullman, Wash. there are usually a considerable number of honey gathering insects flying about when the flowers are in bloom. On the other hand, there are certain localities in the Northwest, where alfalfa seed is produced, sometimes in very large quantities, though honeybees and bumblebees are quite rare.

In the vicinity of Chinook, Montana, several car loads of alfalfa seed were produced in 1908, and also in some other years, though honey gathering insects were almost entirely

absent in most of the fields. This seemed to indicate that, under certain conditions, seed may develop abundantly without the presence of insects to trip the flowers.

In the study of the fertilization of the alfalfa flowers conducted at Chinook and Havre, Mont., in 1909, it was especially desired to find out whether alfalfa flowers sometimes do become automatically tripped, and develop seed without the presence of insects or other outside agency to trip the flowers, or whether the flowers might not develop seed under some conditions without the flowers being tripped. This problem was rendered difficult to solve, however, by the fact that in 1909, in nearly all fields, but a very small proportion of flowers were developing pods, and these pods seemed generally to have been tripped by the limited number of insects which were present, or by some other external means. It was observed, however, that two of the plants enclosed in netting at Chinook, which had been producing a good crop of seed throughout the season, while most of the surrounding plants were producing but very few pods, were producing pods even from those racemes of flowers from which insects had been excluded. Accordingly, all wilted and all unopened flowers were removed from a number of racemes under the netting, leaving only opened, untripped flowers, which were closely observed during the following days.

In a day or two, it was observed that several of the

flowers, from which insects were excluded, had become tripped. On one of these two plants,- plant numbered Montana B,- in a number of the flowers the keel petals were partially separating, evidently being forced apart by pressure of the pistil and stamens from beneath. While these flowers were being examined, one flower was seen to become self tripped. The pistil and stamens snapped up vigorously against the standard, scattering the pollen around, no object had come into contact with any portion of the flower.

The calices of all tripped flowers were marked with carbon ink as soon as they were found to be tripped. The number of flowers that were tripped, and the pods that developed from tripped and untripped flowers, are shown in Table XIII.

Table XIII

Pods and seeds from self-tripped flowers at Chinook, Mont., 1909.

Mont.	Plant number	Raceme number	Number flowers	Number flowers tripped	Percentage of flowers tripped	Total number pods	Number pods from tripped flowers	Number pods from untripped flowers	Percentage of flowers forming pods
"	3	34	12	9	75.00	7	7	0	58.33
"	3	35	11	3	27.27	3	3	0	27.27
"	3	36	7	2	28.57	0	0	0	00.00
"	3	37	11	7	63.63	5	5	0	45.45
"	3	38	10	9	90.00	5	5	0	50.00
"	3	39	6	3	50.00	1	1	0	16.66
			57	33	57.88	21	21	0	36.84
Mont.	8	30	14	5	35.71	3	3	0	21.42
"	8	31	6	2	33.33	0	0	0	00.00
"	8	32	20	14	70.00	8	8	0	40.00
"	8	33	11	2	18.18	0	0	0	00.00
	8	34	13	13	100.00	5	5	0	38.46
			64	36	56.25	16	16	0	25.00

Percentage of all flowers tripped on pls. Mont. 3 and Mont. 8---------57.02
Percentage of all flowers producing seed on pls. Mont. 3 and Mont. 8--30.57
Percentage of tripped flowers producing seed on pls. Mont. 3 and Mont. 8---53.62
Percentage of untripped flowers producing seeds on pls. Mont. 3 and Mont. 8---00.00

The results shown in Table XIII show clearly that alfalfa flowers do sometimes become self-tripped. The figures in this table also show that on these two plants a large percentage of the flowers that were self-tripped produced pods and seed.

By refering to Table IV, it will be observed that in each of the three years in which the pollination experiments which are summarized in this table were carried out, of those flowers which were not tripped when enclosed in mosquito netting, a small percentage produced pods every year.

In 1910, nine alfalfa plants which were producing more pods and seed than most of the alfalfa plants in the grass garden at Pullman, were inclosed in netting tents, and were closely observed for several days to determine whether the small percentage of flowers that frequently produce pods when honey-gathering insects are excluded from the alfalfa plants, become self-tripped, or whether the flowers may produce pods without becoming tripped at all.

The tents in which the plants were enclosed, were carefully covered with fine mesh mosquito-bar, so that there were no openings left for honey-gathering insects

to gain access to the flowers. The tents were made large enough and pains were taken so that no flowers which were being watched were in such a position that they could brush against the sides or top of the tent.

After the flowers from which insects were to be excluded had been enclosed in the netting tents, they were examined every day or every second day until all of the flowers had become entirely wilted. Whenever any flower was found tripped, the calyx was marked with a mixture of carbon-black and water, in order that the pods that developed from tripped flowers might be distinguished from pods that might develop from flowers that had not been observed to be tripped.

Table XlV shows the number of flowers that became tripped and also the number of pods and seed that developed from tripped and untripped flowers.

Table XlV.

Pods and seed from self tripped flowers at Pullman, Wash., 1910.

Plant number	Number flowers	Number flowers tripped	Number flowers not tripped	Number marked pods	Number mature seed from marked pods	Number pods not marked	Mature seed from pods not marked
8	128	9	119	1	0	0	0
9	104	17	87	12	18	4	4
10	59	2	57	1	0	1	0
11	100	15	85	4	3	0	0
12	62	4	58	0	0	0	0
13	91	1	90	1	1	0	0
18	79	3	76	2	4	0	0
19	71	8	63	0	0	0	0
20	81	1	80	0	0	0	0
	775	60	715	21	26	5	4

Percentage of flowers tripped----------7.70

Percentage of tripped flowers producing pods-35.00

Percentage of flowers not tripped producing flowers---------- .69

Mature seeds per pod from tripped flowers---- 1.23

Mature seeds per pod which developed from flowers not observed to be tripped------ .80

Table XlV shows that 7.7 percent of the flowers observed on the nine plants became tripped. In several of the flowers the two petals forming the keel were observed to gradually draw apart, and later the flowers were found to be tripped. As the tents were made so that the honey gathering insects did not have an opportunity to have access to the flowers, as care was taken to prevent any other object from coming into contact with the flowers, it may presumably be assumed that nearly all of the flowers that were tripped were tripped automatically. The evidence obtained from this experiment and also from other experiments conducted at Pullman, Wash. indicate that in most seasons at Pullman, on a large proportion the alfalfa plants, a small percentage of the flowers become self-tripped; and that a considerable proportion of the limited numbers of flowers which become self-tripped produce seed.

In the latter part of August, 1910, alfalfa fields at Chinook, Mont., which were producing seed, were examined, Two fields were found on which pods and seed were developing in umusual abundance. Both fields were profusely in blossom. On August 20 and 21, nearly all flowers that had been open for about one day or more, were tripped. A typical raceme, picked at random was found to have on it twenty opened flowers. Of this number

fifteen were tripped. The five not tripped were all some of the most recently opened flowers located at the tip of the raceme.

An examination of a large number of plants in the field showed that during a period of about two weeks, or possibly for a somewhat longer period preceding August 20th, the majority of the alfalfa flowers had been developing into pods. Prior to this, there was a period during which but a small percentage of the flowers had developed into pods, as was indicated by the stems of the older flower racemes, the majority of which were either without pods, or else had only a small number of pods on them.

On August 22, flowers which were just beginning to open were marked on ten different plants in these two fields and were watched in order to determine what was causing these flowers to become tripped.

Table XV shows the number of flowers that were observed, and the number and percentage of flowers that had become tripped inside and outside of the netting during the four days after the flowers were first observed. Most of the flowers had become wilted by the latter date.

Table XV.

Number of flowers becoming self-tripped in alfalfa fields at Chinook, Mont. 1910.

Inside Netting.

Plant number	Number racemes	Number flowers	Number flowers tripped
9	5	29	0
10	5	48	0
11	6	41	0
12	8	43	0
13	6	37	1
	62	390	1

Percentage of flowers tripped------00.25

Outside Netting.

Plant number	Number racemes	Number flowers	Number flowers tripped
9	5	28	1
10	5	40	2
11	5	36	0
12	7	47	0
13	5	36	1
14	4	35	0
15	6	39	0
16	5	32	0
17	4	18	0
18	4	22	0
	50	333	4

Percentage flowers tripped---------1.2

As Table XV indicates, very few flowers were tripped during the period from August 22 to August 26, either inside or outside of the netting tents; whereas, in the few days preceding August 22, very nearly all flowers in these two fields became tripped after being open for a short time. Prior to August 22, the atmosphere was clear and fairly warm. By August 22, it became much colder; a hard frost occurred on the night of August 24th. During the three or four days following August 22, it was observed that when alfalfa flowers were artificially tripped the pistil and stamens did not snap up against the standard as promptly and vigorously as in the case of those flowers that were artificially tripped during the days preceding August 22.

The only natural external agencies that could have caused the flowers to trip and produce pods so abundantly just prior to August 22 would have been honey-gathering insects, or possibly the action of wind.

The latest date on which there was any appreciable amount of wind, prior to August 22, was on August 18. Flowers were watched, in another field during this wind, the wind was not found to trip any appreciable percentage of the open flowers. During the period of August 19 to 22 inclusive, when nearly all of the flowers which opened became tripped, there was no wind blowing sufficiently strong to cause the plants to sway enough to disturb the

alfalfa flowers. Wind, as a possible agency that might have caused the flowers to be tripped during this period, should, evidently be eliminated.

During the period from August 22 to August 26, several hours were spent in the alfalfa fields each day; during this time not a single wild or domestic honeybee, or a bumblebee was seen. In the more favorable weather conditions that existed during the three or four days preceding about five or six wild bees of the genus Megechile were observed flying about the fields. No domestic honeybees were seen. The honey gathering insects that were present in the large fields of alfalfa, were present in such limited numbers that not more than a very small percentage of the flowers that were tripped during this period, could have been tripped by these insects.

It was clearly demonstrated at Chinook, Montana, in 1909, and at Pullman, Wash., in 1910, that alfalfa flowers do become automatically tripped under certain conditions.

The most plausible explanation for the fact that during the period immediately preceding August 22, nearly all of the alfalfa flowers in these two fields became tripped and developed into pods, while during an earlier period but few pods were produced, and during the period subsequent to August 22 practically no flowers became tripped, seems to be that just prior to August 22 conditions were favorable for alfalfa flowers to become

automatically tripped in large numbers, whereas during a period earlier in the season, as well as during a period following August 22, conditions were unfavorable for the flowers to become tripped in this manner.

Pods and Seed Developing from Untripped Flowers.

Table XlV shows that five of the 775 flowers observed produced pods, when no evidence that these flowers had been tripped could be found. There is a chance for possible error, as some of these flowers may have become tripped without being observed; or the carbon that may have been placed on the calyx may have been removed. However, two of these flowers were observed, in which a pod had begun to develop, and where the tip of the young growing pod pushed through the tip of the keel, while the flower still remained untripped. These two flowers undoubtedly produced pods without having been tripped. It is evidently only in rare instances, however, that a pod and seeds develop from an alfalfa flower which had not been tripped in some manner.

Effect of Tripping Alfalfa Flowers Mechanically.

In 1910, a number of racemes of opened alfalfa flowers on three different plants were clasped between the pages of a notebook. A record was made of the number of flowers that were tripped and which remained untripped, and of the pods and seeds that developed from them. The pods that

developed from tripped flowers were distinguished by marking the calyxes of the tripped flowers with carbon-black.

The object of this experiment was to illustrate the results that might be obtained by tripping alfalfa flowers in the field by any mechanical arrangement that might be devised to do this work.

Table XVl.

Percentage of Flowers Tripped by Clasping Between the Pages of a Book.

Pl. no.	No. race-mes	No. flo.	No. flo. tripp-ed	No. flo. not tripp-ed	Mark-ed pods	No. mature seed from marked pod	No. pods not marked	Number mature seeds from pods not marked
3	4	31	19	12	14	14	1	0
5	4	57	29	28	22	29	3	5
6	5	69	26	43	3	3	0	0
	13	157	74	83	39	48	4	5

Percentage of flowers tripped----------47.1

Percentage of marked flowers producing pods----------52.7

Percentage of unmarked flowers producing pods----------4.8

The results obtained show that 47.1 percent of the flowers were tripped by clasping the racemes between the pages of a book, and that 52.7 percent of the tripped flowers produced pods; and that 4.8 percent of the flowers which were not tripped by this operation produced pods. The figures shown in the table indicate that any mechanical method that might be devised which would trip flowers in alfalfa fields, would, under conditions like those under which this experiment was conducted cause a considerable increase in the total yield of alfalfa seed.

Period During Which Alfalfa Flowers May be Pollinated.

An experiment planned to show at what stages of its development an alfalfa flower may be fertilized, and also to show during how long a period it remains capable of fertilization, was carried on at Pullman, Wash. in 1909. All opened and wilted flowers were removed from a number of racemes on five different plants. On the following day all unopened buds on these racemes were removed, leaving only those flowers which had opened during the preceding thirty-six hours. The flowers had been covered with netting tents in order to exclude insects. As the experiment was carried out in September, when the weather was comparatively cool, the flowers remained fresh and open for a longer period than they would gave remained open in warmer weather. A number of the flowers on these

racemes were tripped each day up to the end of seven days when the tips pf some of the petals were beginning to wilt. The experiment was discontinued at this time for the reason that there were no more flowers left to trip. The number of flowers that were tripped and the percentage of tripped flowers which produced pods are shown in Table X11.

Table X11.

Showing period during which alfalfa flowers may be fertilized.

	Plant 54		Plant 58		Plant 59		Plant 60		Plant 61	
Tripped at the end of	No. flo. tripped	% flo. developing pods	No. flo. tripped	% flo. developing pods	No. flo. tripped	% flo. developing pods	No. flo. tripped	% flo. developing pods	No. flo. tripped	% flo. developing pods
1 day	31	32.25	22	0	12	75.00	18	16.66	20	30.00
2 days			14	21.42	17	64.70	19	15.78	19	5.26
3 days	26	73.07	18	0	19	52.63	16	18.75	17	23.52
4 days			16	6.25	16	37.50	15	26.66	17	58.82
5 days	21	38.09	15	6.66	16	50.00	15	33.33	13	00.00
6 days			5	0	16	68.75	12	33.33	20	65.00
7 days	10	30.00	8	0	9	88.88	14	28.57	16	6.25

A number of flowers were left untripped on these plants. Less than five percent of the untripped flowers produced pods. An examination of Table X11 shows that there was practically no diminution in the ability of the flowers to become fertilized up to the end of a period of seven days after they had opened, or up to about the time when the tips of the petals began to wilt.

EFFICIENCY OF VARIOUS INSECTS IN CAUSING FERTILIZATION OF ALFALFA FLOWERS.

Domestic Honeybees, Bumblebees, and Wild Bees (Megachile spp.)

When alfalfa plants are in blossom at Pullman, Wash., particularly on warm bright days when there is not much wind blowing, honey gathering insects are quite numerous in the alfalfa fields. From observations, it was determined that the most common insects which gather honey from the flowers of alfalfas are the domestic honeybees, (Apis mellifera L.); one or more of several species of bumblebees (Bombus spp.)are usually present; and also a wild bee of the species Megachile. There are a few other species of insects which visit the alfalfa flowers to gather honey, but are present in the fields in only comparatively small numbers.

In the vicinity of Chinook, in northern Montana where alfalfa seed is produced on several hundreds of acres annually, only a very limited number of insects are found in the alfalfa fields when the flowers are in blossom. The insects which are most frequently found are, the domestic honeybee (Apis sp.), bumblebees of different species (Bombus spp.),and one or more species of Megachile.

In 1909 and in 1910, insects were watched while at work getting honey from the alfalfa flowers; a count was

made of the number of flowers which were visited and of the number which were tripped by insects of different kinds. The results of these observations are given in Table XVll.

Table XVll.

Showing percentage of alfalfa flowers tripped by different honey gathering insects.

Date	Species	Where observed	Total number of flowers visited	Number of flowers tripped	Percentage of flowers tripped
1909	Apis sp.--	Pullman,Wash.	318	1	0.31
1910	Apis sp.--	Pullman,Wash.	189	3	1.58
1909	Apis sp.-	Chinook,Mont.	126	6	4.76
1909	Bombus spp-	Havre,Mont.	268	79	29.40
1909	Megachile"-	Pullman,Wash.	52	47	90.38
1909	Megachile"-	Chinook,Mont.	45	42	93.33

Table XVll shows that on 90 percent of the flowers visited by the wild bees of the Megachile spp.were tripped. The bumblebees which were observed were somewhat less effieicnt than the Megachile spp., 29.4 percent of the flowers visited by the bumblebees being tripped. Some of the species of bumblebees appeared to be more efficient than others in tripping the flowers which they visited. Domestic honeybees do not trip more than a small percentage of the flowers from which they gather honey. From the

figures shown in Table XVll, together with the results of additional observations made at Pullman, it seems evident that the domestic honeybees do not ordinarily trip more than about one percent, or less of the flowers that they visit.

The reason why the Megachile species of wild bees trip so much larger proportion of the flowers which they visit than the domestic bees, is apparently due chiefly to the difference in the way that they reach down to the honey secretion with their probosces.

These two kinds of bees are about the same size, and have probosces of about the same length. The bees of the Megachile species reach down directly along the central portion of the standard, and have to push the standard and wing petals apart, to some extent, in order to get to the honey. The force which they exert when obtaining the honey seems to be sufficient to release the mechanism of the flower which holds the pistil and stamens in place, and the flower becomes tripped. The domestic honeybees extend their probosces down to the honey secretion from one side of the central part of the flower. The distance which they have to reach is less than the distance which the bees of the Megachile species have to reach; it is not necessary for the domestic honeybees to force the petals apart to a great extent, and consequently only a very small proportion of the flowers that they visit are tripped

Most species of humblebees have a longer proboscis than either of the two kinds of honeybees which have been described. They approach the flower in such a way, however, that a large proportion of the flowers are tripped. The comparatively heavy weight of these insects,- particularly of some of the larger species, appears to have some influence in forcing the petals of the flowers apart.

A count was made in 1907 of the number of flowers which several different bees of the species of Megachile common at Pullman, Wash., tripped within a definite period of time. One of these bees tripped 4 flowers within 30 seconds; another tripped 12 flowers in one minute and 10 seconds; while a third tripped 20 flowers in 2 minutes and 15 seconds. These three bees tripped the flowers at the rate of an average number of 9.2 flowers per minute or 552 flowers per hour. A single one of these wild bees is capable of fertilizing a very large number of flowers in one season, and if present in sufficient numbers would be an important factor in fluencing the development of the seed crop. At Pullman, Wash., a large proportion of the alfalfa flowers which produced seed in the experimental plots, during the four seasons in which this study was carried on there, were fertilized by these insects. In the vicinity of Chinook, Montana, the numbers of these bees or of any other honey-gathering insects which are found there, and which trip the flowers that

they visit, are much too small to fertilize more than a small proportion of the flowers which produce seed.

Thrips

Thrips (Enthrips tritici) were present in large numbers in the alfalfa flowers at Pullman,and in less numbers at Chinook, and Havre, Montana. At Pullman, 1119 thrips were found on 16 racemes of alfalfa flowers, or an average of 69.9 thrips on the flowers of each raceme. At Havre 48 thrips were found on 13 racemes. These minute insects do not trip the alfalfa flowers. They were abundant on practically all of the plants used in carrying out the experiments which have been described in this report. As only a very small percentage of the flowers which were not at Pullman produced seed, and as the flowers that were tripped produced seed abundantly, it may be assumed that the thrips are neither appreciably beneficial or injurious in their influence upon the development of alfalfa seed.

Night-flying Insects.

An experiment was conducted at Pullman in 1909, to obtain information in regard to whether night-flying insects are of any importance in fertilizing alfalfa flowers. Seven plants were enclosed in fine meshed mosquito netting. Five of these plants were left under the netting during the entire time of the experiment in order

to find out what proportion of the flowers become tripped when insects are entirely excluded. Two of the plants were kept enclosed in netting during the daytime, but were uncovered during the night. The results obtained are given in Table XVlll.

Table XVlll.

Showing efficiency of night flying insects.

Plant number	Enclosed in netting during entire experiment. Percentage of flowers tripped	Enclosed in netting during entire experiment. Percentage of flowers developing pods	Percentage of flowers developing pods on same plants outside of the netting- open to insects
11	0	1.16	37.31
12	0	0	16.12
14	3.92	3.57	56.60
15	3.03	2.59	20.51
17	0	1.36	
	Enclosed in netting in daytime; open for night-flying insects		
7	4.63	5.78	35.38
10	4.60	2.51	30.35

The results of the experiment show that when insects were excluded from the plants, the percentage of flowers from which pods developed was very much less than from the flowers on the same plants which were left open to the access of insects. The percentage of flowers that were tripped, and the percentage of flowers from which pods developed was only very slightly greater on those plants which were open to the access of insects at night, than on the plants from which insects were excluded during the entire time of the experiment. The results obtained indicate that night-flying insects are of practically no importance, as a factor influencing the production of alfalfa seed at Pullman.

VARIATION IN THE PERCENTAGE OF FLOWERS PRODUCING PODS, AND NUMBER OF SEEDS PER POD, ON DIFFERENT PLANTS.

In carrying out the experiments conducted at Pullman and Chinook, it was observed that there was usually a considerable variation in the percentage of flowers producing pods, and in the number of seeds per pod, on different plants which had received like treatment. This variation is shown in Table XVlll, which gives the results of an experiment conducted at Pullman in 1908. The first position of the table gives the percentage of flowers producing pods, and the number of seeds per pod, produced by flowers which developed under natural conditions on seven different plants. The variation in the percentage of flowers producing pods is partially due,- no doubt, to different numbers of flowers becoming tripped by insects on the different plants.

The second portion of the table shows the percentage of pods, and the number of seeds per pod, produced by flowers from which insects had been excluded. The pods and seeds which developed apparently developed from self-tripped flowers.

The third portion of the table shows the variation in the percentage of flowers which produced pods, and the number of seeds per pod, which developed on the same seven plants, from flowers which were artificially tripped.

Table XVlll.

Variation in Seed Production in Alfalfa Plants.

Not enclosed in netting; flowers developed under natural conditions.

Plant number	Total no. of flowers	No. pods developed	No. of seeds	Percentage of flowers developing pods	Number of seeds per pod
46	59	7	6	11.8	.85
83a	42	18	39	42.8	2.16
83	37	15	42	40.0	2.80
40	171	28	67	16.3	2.39
40a	135	20	25	14.9	1.25
76	68	9	23	13.2	2.55
09	121	58	224	40.5	3.80

Enclosed in netting; not tripped.

46	92	0	0	0.0	0.00
83a	50	4	2	8.0	0.50
83	60	1	0	1.6	0.00
40	143	4	4	2.8	1.00
40a	133	9	7	6.7	0.77
76	59	0	0	0.0	0.00
09	114	7	16	6.1	2.30

Enclosed in netting; tripped artificially.

46	81	1	0	1.2	0.00
83a	35	7	8	20.0	1.14
83	68	18	23	26.4	1.27
40	134	60	88	44.7	1.46
40a	136	52	75	38.2	1.44
76	57	3	3	5.2	1.00
09	65	7	8	10.7	1.14

SUMMARY

Of the flowers observed on a large number of alfalfa plants at Pullman, Wash., and at Chinook and Havre, Mont., in 1908, 1909 and 1910, 23.5 percent of those flowers which developed under natural conditions produced seed. When honey-gathering insects were excluded by means of fine-meshed mosquito netting, the number of flowers producing seed was reduced to 7.57 percent. Of those flowers from which insects were excluded, which were artificially tripped, 36.07 percent produced seed. The pods which developed from flowers which were open to the access of insects contained an average number of 3.09 seeds each; the pods developing from flowers from which insects were excluded contained 2.24 seeds each, and the flowers artificially tripped produced pods containing 2.22 seeds each.

When alfalfa flowers were protected from the sun and wind by one thickness of mosquito netting but were accessible to insects, the percentage of flowers which produced pods and seed was not appreciably different than the percentage of flowers producing pods and seed when conditions were natural. These results indicate that the single thickness of mosquito netting which was placed on a portion or all of the plants in the various experiments

conducted, did not have any effect except by excluding insects, upon the development of the flowers under it.

In 1908, flowers on a number of different alfalfa plants were enclosed in netting; a portion of the flowers had been naturally tripped before they were enclosed in netting, while other flowers had not been tripped. Of the flowers which were tripped before insects were excluded, 58 percent produced seed. Of the flowers which were not tripped at the time insects were excluded, 5.2 percent produced seed. The pods developing from the flowers which had been tripped contained 3.53 seeds each, while the pods developing from the flowers which were not tripped when enclosed in netting contained 1 seed each. The results of this experiment agree substantially with the results of other series of experiments, in showing that at Pullman, Wash., pods and seed ordinarily develop in large numbers only when the flowers have been tripped by insects or some other agency; but that even when insects have been excluded a comparatively small percentage of the flowers produce seed. The results of this experiment also indicate that a larger number of seeds per pod develop when the flowers have been tripped by insects, than when the flowers have been tripped under conditions where there is not an opportunity for the flowers to become fertilized with pollen from other plants.

In 1909 an experiment was conducted for the purpose of determining the comparative effect of pollen obtained from the flowers of a different plant, from other flowers of the same plant, and of pollen from the same flower, in fertilizing flowers that were artificially tripped. In one experiment conducted in 1909, 59.09 percent of the flowers which had been fertilized with pollen from other plants, produced pods containing 3.71 seeds each; 32.16 percent of the flowers fertilized with pollen from the same flowers produced pods containing 1.56 seeds each. In another experiment carried out in the same year, 67.37 percent of the flowers fertilized with pollen from other plants produced pods containing 3.42 seeds each, and 42.85 percent of the flowers fertilized with pollen from the same flowers produced pods containing 1.61 seeds each. In 1910, 34.8 percent of the flowers which were fertilized with pollen from other plants produced pods containing 1.87 mature seeds each; and 23.5 percent of the flowers which were fertilized with pollen of other flowers of the same plant produced pods containing 1.3 mature seeds each.

The results of these three series of experiments showed that when alfalfa flowers were fertilized with pollen from other plants, a larger percentage of the flowers produced pods and a larger number of seeds per pod developed than when the flowers were fertilized either with pollen from the same flower or from other flowers on the same

In an experiment conducted in 1910, there was not any marked difference in the results obtained by fertilizing flowers with pollen from the same flowers or with pollen from other flowers of the same plant.

In another experiment carried out in 1910, the percentage of pods developing from flowers which were tripped without having the stigma or anthers come into contact with any object, was compared with the number of flowers producing pods under natural conditions. 52.24 percent of the flowers which were tripped naturally, probably by insects, produced pods. Of the flowers from which the standards were removed, and which were tripped so that the pistil and stamens did not come into contact with any object during the process, 14.15 percent produced pods. When the flower was tripped by cutting the base of the keel petals in such a way that the pistil and stamens moved up to the standard, without emerging from the tip of the keel, 13.16 percent of the flowers tripped in this way produced pods. When the flowers were artificially tripped with a piece of alfalfa stem, using one piece of stem for all the flowers on one raceme, 41.73 percent of the flowers produced pods. The results of this experiment indicate that the mechanical action upon the surface of the stigma, which is brought about when it

strikes upon the object tripping the flower or against the standard has some influrnce in causing fertilization. This effect may be brought about either by bringing the pollen into contact with the surface of the stigma or by irritating the surface cells of the stigma, or to both of these causes.

Alfalfa plants were observed at Pullman, Wash. and at Chinook, Mont., in 1909 and 1910, to determine whether pods and seed ever develop from flowers which have been automatically tripped. At Chinook, in 1909, two plants were found on which the flowers were becoming self-tripped in large numbers. On one of these plants 57.88 percent of the flowers and on the other plant 56.25 percent of the flowers observed were automatically tripped. At Pullman, Wash. in 1910, 7.7 percent of the flowers enclosed under netting were self tripped. At Chinook, Montana in 1910, nearly all of the flowers which opened during a period of two weeks or more early in August became tripped, when there were not enough insects in the fields to trip more than a small proportion of the flowers; in the few days succeeding this period, when climatic conditions were changed, practically none of the flowers were tripped. The results of these observations indicate that usually a small percentage of the alfalfa flowers

become automatically tripped; and that under some conditions, the flowers on a portion or all of the alfalfa plants become self-tripped in large numbers. Observations made at Chinook, Montana lead the writer to the conclusion that the large crops of alfalfa seed which are produced there in certain seasons, develop chiefly from flowers which have become automatically tripped.

Observations made at Pullman, indicate that in comparatively rare instances, alfalfa flowers which have not been tripped may produce pods. However, the numbers of pods and seed developing from untripped flowers evidently is very small.

Alfalfa flowers on a number of racemes were tripped by clasping the racemes between the pages of a book. 47.1 percent of the flowers on these racemes were tripped in this way, and 52.7 percent of the flowers which were tripped produced pods. The results of this experiment indicate that the yield of alfalfa seed might be increased when insects are not present in the fields in sufficient numbers to trip the flowers, by any mechanical arrangement that might be devised to trip the flowers.

An experiment was conducted at Pullman, Wash. in September 1909, to determine for how long a period alfalfa flowers remain susceptible to fertilization. It was

found that up to the time when the experiment ended, seven days after the flowers opened, when the tips of the petals were beginning to wilt, there was practically no diminution in the ability of the flowers tripped to become fertilized.

The percentage of flowers visited which are tripped by different kinds of honey-gathering insects varies greatly. It was found that domestic honey bees usually trip only about one percent or less of the flowers. Different species of bumblebees vary in their efficiency in tripping flowers; at Havre, Mont., several different species which were observed tripped an average of 29.4 percent of the flowers that they visited for honey. Wild bees of the Megachile species tripped over 90 percent of the flowers which they were observed to visit. Observations of the small thrips which were common on the alfalfa flowers in Washington and Montana, leads to the conculsion that these insects neither appreciably increase or decrease their percentage of flowers producing pods and seed. An experiment conducted at Pullman, Wash. indicates that night-flying insects are not an important factor in influencing the development of the alfalfa seed crop.

It was found that there is a considerable amount of variation in the percentage of flowers producing pods and in the number of seeds per pods, on different alfalfa

Appendix

Historical Resume'

(Verhandulungen Des Botanischen Vereins 1873, page 13 by Dr. I. Urban)

The tripping mechanism of alfalfa flowers has been known to a limited number of biological investigators for a considerable time.

The first reference that we have to the explosive mechanism in the flowers of Medicago sativa and of other leguminous plants is a statement by Urban, that the tripping of these flowers was known in Linneus' time.

Medicago Flowers Tripping Historical Resume'

(Physiologie Vegetable Vol. 2 p 548 A.P. deCondolle 1832)

In 1832 in his book on The Physiology of Plants, de Candolle gave a brief description of the tripping phenomna He said "Certain corolla contribute in an indirect way to fertilization; thus the pieces of the corolla of Indigofera and some lucernes are fixed one to the other by a kind a hook. When their development is accomplished these hooks are detached; the carina no longer fixed is warped elastically and stamps a blow upon the sheaf of stamens which determine the fall of the pollen."

Medicago Flowers Tripping Historical Resume'

De Candolle: Pflanzen-Physiologie: p. 116 Aus dem Franzsischen ubersetzt von Johannes Roper, 1835, p. 116-111. Buch. Par. 6.

In this translation into the German language Roper gives the same statement in regard to the tripping mechanism in Medicago that deCondolle originally stated in his original book on The Physiology of Plants written in French and published in 1832.

Medicago Flowers Tripping Historical Resume'

Journal of the Linnean Society of London 1867 p. 328 Rev. Geo. Henslow.

In a paper read at a meeting of the Linnean Society in Nov. 1865 Henslow discussed the results of his observations and experiments with flowers of alfalfa and other plants.

Henslow was the first, according to any available record, who studied carefully the explosive mechanism of alfalfa flowers and attempted to explain its cause. He attributed the elasticity of the staminal column to the turgidity of the cells of the nine cohering filiments of the staminal tube. He found that the tenth or free staymen appeared to have no such elastic property. When the pistil was removed from the tube it exhibited no elasticncy to become curved. On the other hand

when the staminal tube had once become tripped he found that he could not straighten it to its original position without causing a transverse fracture.

Henslow also observed hive bees sucking the nectar from the flowers of Medicago sativa. The flowers were frequently visited by the bees but in no instance did he find that they had power enough to set free the staminal tube. No opportunity of observing bumblebees presented itself.

Medicago Flowers Tripping Resume'

(Botanische Zeitung jahrg. 24 (1866) Von F. H. Hildebrand P 74)

In an article published in 1866 Hildebrand discusses the result of his studies of the flowers of Medicago and other plants.

He gives a description of alfalfa flowers in their explosive arrangement. He quotes Triviranus' statement to the effect that flowers in Indigofera become tripped naturally. Hildebrand states that flowers in species of Medicago behave in the same way.

According to a statement made in 1894 by Burkhill, Hildebrand asserted that the flowers of M. sativa is fertile if allowed to remain unexploded.
(I.H. Burkhill Proceedings of the Cambridge Philosophical Society for 1894 p. 142)

Medicago Flowers Tripping Historical Resume'

(Botanische Zeitung jahrg. 25 (1867) Von F. H. Hildebrand P 283.)

In 1867 Hildebrand discusses the results of Delpino's study of the blossoms of different species of Medicago. Delpino described the alfalfa flower and its tripping mechanism but evidently made no important addition to what was already known on this subject.

Medicago Flowers Tripping Historical Resume'

Dr. I. Urban; Verhandlung des botanischen Veresns der Provinz Brandenburg: 1872, p. 13ff.

Urban states that the peculiarity which the Medicago species show in their pollination, namely that the stamen is propelled out of the keel toward the standard when the keel is pressed on, or a needle inserted, was known in the time of Linneaeus, but its significance was not understood. De Candolle believes that this movement of the individual parts of the flower is for the purpose of bringing about direct self fertilization.

"When the corolla barely reaches above the calyx teeth a decided tension develops in the side of the stamen tube that is turned towards the keel."

"Before pollination the standard lies close to the wing, so that it leaves only a narrow channel open which leads somewhat obliquely to the nectar chambers between the points on the wing just mentioned. These nectar chambers lie at the base of the stamen tube on the inner

side. They are roll shaped projections, and can be reached from the base of the tube through two clefts, which are found on either side of the free stamens."

"When the bloom reaches complete development the tension in the stamen tube ceases so that the stamens remain closed in the keel only in a few cases have I found fertilized ovules in such cases."

According to my observations, which have been made frequently and at all times of the day, only bees bring about pollination, though butterflies enjoy the flowers just as well; but they can not push aside the points on the wings, and it is not necessary, since their more flexible proboscis can reach around them to the nectarium."

"All these arrangements for pollination are not limited to the genus Medicago; besides the small flowered Medicagos, there are numerous Trigonella species, for example Fischerianna, Pinnatifida, Polycerata, etc., while others, for example Corniculata, Calliceras, etc., and Pocockia cretica have a pollination apparatus like that described by Hildebrand for Indigofera."

Medicago Flowers Tripping Historical Resume'

(The Fertilization of Flowers by Hermann Muller pars. 93 & 94 1873.)

Muller gives a detailed description of the alfalfa flower and its explosive mechanism. He states that in Medicago sativa the elastic tension resides almost exclusively in the upper stamens. In Sarothamnus scoparius which also has explosive flowers, it is the style only which acts as a spring; in Genista tinctoria the column tends to spring upwards and the alae and carina together tend to spring downwards.

Muller says : "self fertilization is undoubtedly efficient for Hildebrand has shown that flowers which wither unexploded when insects are excluded produce seed

by self fertilization."

Muller states that : "although hive bees are by far the most numerous visitors of this flower, I have never seen them effect explosion; but I have often observed them. Though I have frequently watched this plant I have never succeeded in seeing explosion actually performed though the number of exploded flowers that we meet with shows that it must take place very frequently. Butterflies visit the flowers in great numbers and it is doubtless by them that explosion and cross fertilization are effected; but they are generally too wild to permit close observation of their movements."

Medicago Flowers Tripping Historical Resume'

(Proceedings of the Cambridge Philosophical Society 1894 p. 142, I.H. Burkhill)

At the meeting of the society named above on Feb. 12, 1894, Burkhill read a report of his quite extensive study. The subject of his paper was ; On the Fertilization of Some Species of Medicago in England.

He first gave a historical resume' of the work that had already been done by different investigators and included a bibliographical reference to the published results of their work.

Burkhill gives a detailed description of the alfalfa flower and its explosive mechanism. He states; "the explosive action of the flower depends upon the uppermost

stamens of the fused nine : these by having the cells of their filiaments intensely turgid tend to make the whole staminal tube assume a curved form whereby the stigma and anthers are forced against the vexillum. This explosive force is resisted by the paired combining processes of the alae and carina and according to my observation not by the basal process of the alae."

Burkhill covered a considerable number of alfalfa flowers with nets to prevent insect visits: no seeds are set he states in the unexploded flowers.

Burkhill states that the stigma does not become receptive until rubbed or until its cells are injured in some manner. He says: "My proof is I think conclusive: Firstly, the stigma appears not to be moist, but when rubbed on glass leaves a sticky mark. Secondly, I have caused flowers to set seed though unexploded, (1) by pinching the stigma through the kell, (2) by perforating the keel with a needle and scratching the stigma, and (3) by cutting off the tip of the keel and rubbing the stigma with a stiff paint brush."

Burkhill says that the hive bee does not explode the flower, but inserts its probosis obliquely over the basal processes and not between them. It is necessary to produce explosion for an insect to insert its probosis between these basal processes.

Burkhill found that it required an average weight of 1.68 grammes to explode a flower of Medicago sativa (Maximum and minumum 2.37 and .93) The worker of Apis he found to weigh about .096 and Bombus hortorum about .199 grammes. The mere weight of these two insects is therefore according to Burkhill, insufficient to explode the flower.

Burkhill says the flower is not always in the same degree of explosiveness; the hotter the weather the more explosive is the flower.

He says shaking by the wind cannot explode the flowers Pieces of paper with a surface of 18 1/2 and 22 square inches were tied to stalks of this plant in order to give more power to the wind but no effect was observable from the shaking it produced.

Medicago Flowers Tripping Historical Resume'

(Contribution from the Entomological Laboratory; No. 65; Univ. of Kansas S.J. Hunter, 1898)

Hunter published a bulletin in which he discussed alfalfa and honey bees. He describes the tripping mechanism of the flowers. He evidently assumed that the common domestic honey bee is capable of tripping the flowers but does not record any observations of his own.

Hunter gathered a large number of representative ripened pods from an alfalfa field less than one half mile

away from a large apiary, and a like number from another field of much the same soil and, practically, under like conditions as the first field except that the second field was situated twenty-five miles away from a colony of bees. No bees were observed in the latter field. The pods from each field were carefully opened and the number of seeds in each counted. 87 pods taken half a mile from the bee hives contained 482 seeds or 5.58 per pod. 80 pods taken 25 miles from these produced 268 seeds or 3.35 per pod.

Medicago Flowers Tripping Historical Resume'

(Kirchner, Q. On the effect of self fertilization in Papilionaceae. Naturw. zeitschrift fur land- und forst-wirthschaf. January 1905. heft. 1-3, p. 45.)

Kirchner states that the reports on the success of self fertilization of the violet blossoms of Medicago sativa which are provided with an explosive arrangement are contradictory.

"The experiments made by us", he states, "in 1902, at Hohenheim, showed plainly that the plants are self stirile.. . . Fifty-four exposed clusters of blossoms on two plants with 432 blossoms produced Aug. 23, 208 pods which, though they were not perfectly ripe, showed plainly that they contained 636 well developed seeds. On the other hand 21 covered closters of blossoms on the same plant with 166 blossoms produced only 2 pods with 3 seeds."

"Similar results were also obtained with the flowers

of Medicago falcata. Of one hundred and four exposed clusters of blossoms with 940 blossoms, 152 pods were produced with 177 seeds, whereas 206 covered clusters with 1882 blossoms produced no seeds at all. As the pistils are closely surrounded by the stamen, spontaneous self fertilization must take place, but without effect."

Kirchner carried out experiments with the flowers of leguminous plants during the years of 1900 to 1904. He classified them as either self fertile or self sterile. He classifies both Medicago sativa and M. falcata among the self sterile species.

Medicago Flowers Tripping Historical Resume'

After Hunters report on the relation of insects to alfalfa which was published in 1898 the next discussion of this subject, which is recorded in this country, is by Mr. J. M. Westgate, of the United States Department of Agriculture in a paper presented at the American Breeders' Association at Lincoln, Nebraska, Jan. 17 to 19, 1906. He presented a brief review of the work of Henslow, Urban, Kirchner and Burkhill. He pointed out the general disagreements between the results that have been obtained by different investigators. He suggested that a great deal of work needs to be done on the subject of the fertilization of alfalfa with the object of determining to what

extent the stigma is sterile to the pollen of its own flower, of the same cluster and of other flower clusters of the same plant.

Medicago Flowers Tripping Historical Resume'

(Bulletin No. 151, Kansas Experiment Station H. F. Roberts and G. F. Freeman, Dec. 1907.)

Roberts and Freeman give the results of some experiments to determine the effect of close pollination. Plants were inclosed in wire netting tents. A portion of the flowers were pollinated by hand and a portion were left without pollination. On the plant on which this experiment was carried out 59 seeds were produced from flowers which were not hand pollinated and 118 seeds were produced from the hand pollinated flowers. In another experiment with five different plants which were producing seed abundantly, wire netting cages were placed across the middle of the plant thereby enclosing the half on which the flowers were to be hand pollinated within the cage and leaving the remaining half standing out side the cage freely accessible to insect visitors.

Far more pods and even more pods containing seeds were produced on the hand pollinated series but a greater number of seeds per pod were produced in the insect pollinated series and as a matter of fact more seeds per plant on this account. Taking the green weight of the

plant as a basis of comparison, the insect pollinated series gave 29.7 seeds per each ten grammms of plant weight while the hand pollinated section produced slightly less, - 25.9 seeds per 10 gramms of weight of the plant.

Seven different alfalfa plants were inclosed within wire netting cages; the number of seeds which developed while the plants were inclosed under netting were later counted. The results obtained are given in the following table. The flowers were artificially tripped.

Plant No.	Apparent seeding ability	Total number seeds	No. of seeds per 10 gramms green weight of plant.
69	Strong	27	1.72
71	Weak	1	0.14
89	Strong	214	20.57
90	Strong	257	21.24
91	Weak	23	0.73
92	Strong	59	3.31
70	Strong	25	2.40

Roberts and Freeman describe a method of exploding flowers in large numbers by rolling the head carefully but firmly between the thumb and the first and second fingers.

Medicago Flowers Tripping Historical Resume'

(Circ. 24, B.P.I. U.S. Dept: of Agri. C.J. Brand & J.M. Westgate, March 5, 1909.)

A very brief discussion of the relation of insects to the setting of alfalfa seeds is given in this bulletin.

The authors state that insect visits are essential to the proper pollination of the alfalfa flowers. They state that bumble bees are the most efficient of all insects in setting off the explosive mechanism and hence in bringing about pollination. Honey bees though not nearly so effective as bumble bees should not be under rated in this connection. Wild bees (Andrena spp. and Megachile spp.) and various butterflies are also valuable agents in pollinating alfalfa flowers.

A brief discussion of results obtained at Arlington Experimental Farm and at Chico, California by the U. S. Dept. of Agri. is given. The results show that when alfalfa flowers were artificially pollinated on the Arlington Farm Twenty-five and one half per cent more pods were produced than when the flowers were not artificially pollinated. At Chico, Calif. a row of alfalfa plants artificially pollinated produced 129% more pods than the plants in the row not artificially pollinated.

Medicago Flowers Tripping Historical Resume'

(Report of American Breeders' Association, 1909)

A report of questions submitted to men engaged in alfalfa breeding in different parts of the United States, is given by the committee in charge of breeding forage crops of which Prof. C. V. Piper was chairman. The opinion of the different alfalfa breeders, in regard to the

importance of insects and of other agencies which may trip the alfalfa flowers is given here. The opinion of two or three of these men in regard to whether alfalfa flowers may become self tripped or whether pods may develop without the flowers being tripped at all is also given.